Responses of barley *Albina* and *Xantha* mutants deficient in magnesium chelatase to soil salinity

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ABSTRACT

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Soil salinity reduces the plant growth and grain yield in barley. The barley mutants *Albina* and *Xantha*, deficient in magnesium chelatase, represent a suitable model object for analysis of the roles of chloroplast in salt stress response. Spring barley (*Hordeum vulgare* cv. Svalofs Bonus) and four nonallelic *Albina* (*alb-e[16]* and *alb-f[17]*) and *Xantha* (*xan-s[46]* and *xan-b[12]*) mutants were used to investigate the effects of soil salinity on physiological traits of plants. Under salt stress, larger reduction in stomatal conductance and higher Na concentration was found in *Albina* and *Xantha* mutants compared with wild type (WT). In addition, the *Albina* and *Xantha* mutants had lower capacity of reactive oxygen species (ROS) scavenging while higher ROS generation rate compared with WT, exposed to soil salinity. Therefore, the limitations in chloroplast development affected Na^+/K^+ homeostasis and decreased the oxygen scavenging capacity, hence affecting the salt tolerance in barley.

Keywords: stress condition; ion toxicity; chlorophyll biosynthesis; sugars

Soil salinity is an important limiting factor in crop production, which is predicted to affect approximately 800 million hectares of global arable land (Janda et al. 2016, Zhang et al. 2016). Barley (*Hordeum vulgare* L.) is grown in a wide geographic range. However, in many regions, farmers have to irrigate their land with saline water due to the shortage of fresh water (Wang et al. 2015). High salt stress reduces the growth rate of barley plants, increases leaf senescence and reduces tillering, leading to a significant grain yield loss (Pérez-Lopéz et al. 2013). Salt stress has been also reported to cause chloroplast damage, decrease photosynthetic carbon assimilation, in-

crease photorespiration and induce accumulation of reactive oxygen species (ROS) (Wu et al. 2014, Chen et al. 2015). ROS are generated in the process of photosynthesis in chloroplasts, especially under the stress conditions such as salt (Li et al. 2015). The effects of salt stress on barley crops are due to osmotic stress and the ion toxicity of Na^+ and Cl^- (Nevo and Chen 2010). According to these, the mechanism of plant response to salt stress is a complex phenomenon, which involves the processes at cellular, biochemical and whole plant levels.

Maintenance of Na^+/K^+ homeostasis and ROS scavenging are two important strategies for alleviating oxidative damage and achieving higher salt tolerance for wheat crops (Zhang et al. 2016). Chloroplasts are important cellular organelles with the main function of conducting photosynthesis (Li et al. 2014); furthermore the antioxidant enzyme system in chloroplasts plays a key role in ROS scavenging, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Thus, chloroplast may be important for the tolerance of plants to soil salinity.

Chloroplast development and photosynthesis depend on chlorophyll biosynthesis, which is a complex stepwise process (Gálová et al. 2000, Müller and Hansson 2009, Bresic et al. 2015, 2016). Magnesium chelatase, the first committed enzyme in the chlorophyll biosynthesis, is important for plant development and stress responses (Müller and Hansson 2009). The chlorophyll mutants were used as a suitable model object for analysis of abiotic stress response, because they respond very sensitively to abiotic stress (Gálová et al. 2000). In this study, four nonallelic *Albina* (*alb-e16* and *alb-f17*) and *Xantha* (*xan-s46* and *xan-b12*) mutants were selected and analysed to represent four distinct steps of the chloroplast development (Campoli et al. 2009). Chloroplasts are critically important cellular organelles related to the early responses of plants to salt stress, as most of ROS and reactive nitrogen species (RNS) are generated during photosynthesis in chloroplasts, especially in salt stressed seedlings (Zheng et al. 2017). The *Albina* and *Xantha* barley plants carrying a mutation preventing chloroplast development are suitable for analysis of the roles of chloroplast in salt stress response. Although these mutations are lethal, the large seed endosperm can support plant growth for several weeks, allowing studies

on these mutants (Dal Bosco et al. 2003, Olsson et al. 2004, Rzeznicka et al. 2005, Svensson et al. 2006, Campoli et al. 2009, Müller and Hansson 2009). To investigate the roles of chloroplast development in formation of salt tolerance in barley, the nonallelic *Albina* and *Xantha* mutants were exposed to soil salinity (200 mmol) for 6 days and the stomatal performance, Na^+/K^+ homeostasis and ROS metabolism were analysed. The hypotheses were that: (1) the sensitivity of barley plants to soil salinity is higher in *Albina* and *Xantha* mutants compared with wild type; (2) the limitations in chloroplast development alter Na^+/K^+ homeostasis and decrease the oxygen scavenging capacity, hence affecting the salt tolerance in barley.

MATERIAL AND METHODS

Genetic materials and experimental setup. Spring barley (*Hordeum vulgare* cv. Svalofs Bonus) and four nonallelic *Albina* (*alb-e16* and *alb-f17*) and *Xantha* (*xan-s46* and *xan-b12*) mutants were used in the pot experiment. These mutations influencing chloroplast development were located in magnesium chelatase, the first committed enzyme in the chloroplast biogenesis pathway (Hess et al. 1992). All mutants were maintained as heterozygous stocks and a 3:1 segregation was observed after germination (Svensson et al. 2006). Wild type (WT) and mutants were grown in pots filled with peat in a climatic chamber for 14 days at 20°C. Then half of the wild type and mutant plants were irrigated with a solution (water) containing 200 mmol NaCl for 6 days as a salt treatment. The remaining pots were irrigated with water as control. The experiment was a randomized block design with three replicates. Each replication consisted of fifteen plants.

Physiological trait determination. Stomatal conductance (g_s) was measured with a leaf porometer (Decagon Devices, Pullman, USA) after salt treatment. The plant height, root length and dry weight were determined just after salt treatment. Plants were also sampled into liquid nitrogen and stored at –80°C for further analyses. The concentrations of Na and K in leaf were measured with a TAS 986 atomic absorption spectrophotometer (Beijing Purkinje General Instrument, Beijing, China). Leaf H_2O_2 concentration was determined by monitoring the absorbance of titanium per-

oxide complex at 410 nm as described by Li et al. (2013). The SOD activity was determined by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium, the activity of APX was assayed following ascorbate oxidation by monitoring the decrease at 290 nm, and the CAT activity was measured following the methods of Li et al. (2014). Concentrations of total soluble sugar, sucrose and free amino acid in dry leaf samples were determined according to Zhang et al. (2011) and Li et al. (2013).

Statistical analysis. All data were firstly tested for homogeneity of variance with boxplot and then subjected to two-way ANOVA to determine the significant differences between salt treatments and mutants using the software of SigmaSATA (V3.5, Systat Software, San Jose, USA).

RESULTS AND DISCUSSION

Compared with their respective control, the plant height (PH) was reduced by 14.7% and 24.0–34.3% under salt in WT and four mutants (Figure 1). However, the root length (RL) was decreased by 38.9% and 7.7–24.1% under salt in WT and four mutants, compared with their respective controls. Salt treatment reduced the dry weight (DW) by 19.0% and 3.1–17.3% in WT and four mutants, compared with the control, respectively. Also, two-way ANOVA showed that there was a significant difference between WT and mutants in PH and DW, respectively (Table 1). It should be noted that the salt-induced reduction in plant height was higher in *Albina* (*alb-e16* and *alb-f17*) and *Xantha* (*xan-s46* and *xan-b12*) mutants than that in WT. This indicated that the negative effect of soil salinity was larger in the mutants, due to the depressed chloroplast development. No significant difference was found in root length between WT
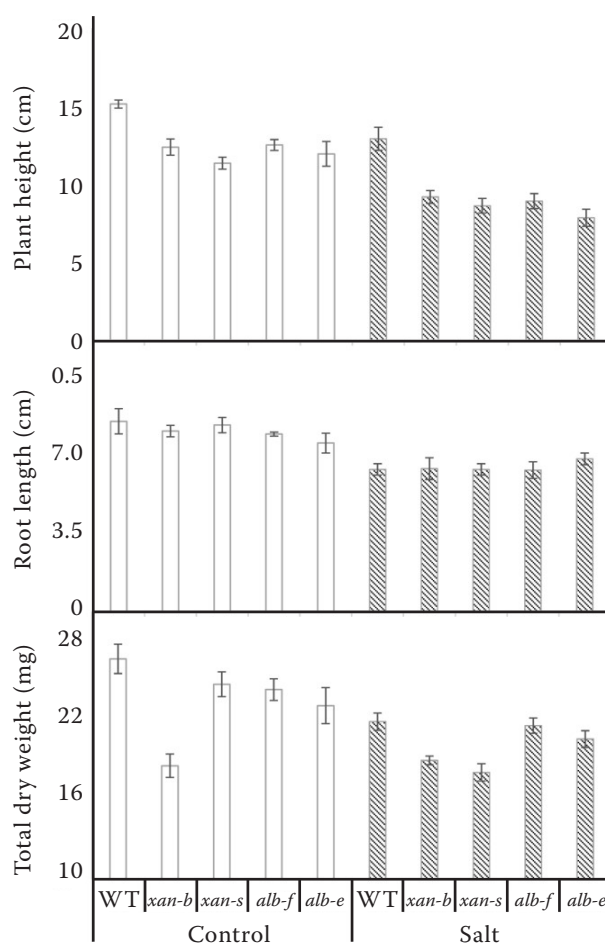

Figure 1. Effects of salt stress on plant height, root length and total dry weight in barley mutants *Albina* and *Xantha*. Mean values ± standard error are shown (*n* = 6). WT – wild type

and mutants either under the control or under the salt treatment. Müller and Hansson (2009) documented that there was no significant difference between WT and the *Albina* and *Xantha* mutants under abscisic acid treatment and the control (pure water). Under the present conditions, interestingly the dry weight of *Xantha* mutants (*xan-s46* and

Table 1. Output of statistical analysis on the traits of barley mutants *Albina* and *Xantha* under salt stress

Factor	PH	RL	DW	g_s	Na	K	H_2O_2	SOD	APX	CAT	TSS	Sucrose	FAA
P_S	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.574	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P_M	< 0.001	0.912	< 0.001	< 0.001	0.007	< 0.001	0.040	0.029	0.168	< 0.001	0.001	0.335	< 0.001
$P_{S \times M}$	0.445	0.331	0.116	< 0.001	0.012	0.207	0.068	0.002	0.325	< 0.001	0.473	0.903	0.014

PH – plant height; RL – root length; DW – total dry weight; g_s – stomatal conductance; SOD – superoxide dismutase; APX – ascorbate peroxidase; CAT – catalase; TSS – total soluble sugar; FAA – free amino acid; P_S – *P*-value of salt effect; P_M – *P*-value of mutant effect; $P_{S \times M}$ – *P*-value of the interaction of salt by mutant
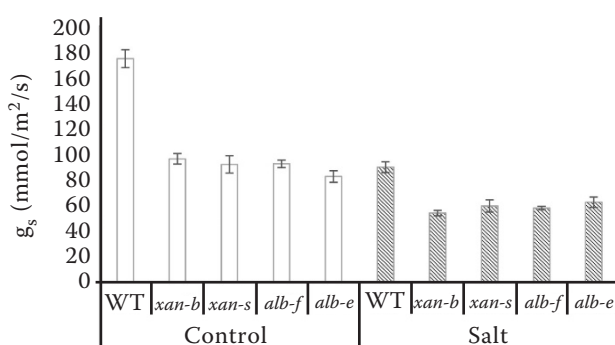
Figure 2. Effects of salt stress on stomatal conductance (g_s) in barley mutants *Albina* and *Xantha*. Mean values ± standard error are shown (*n* = 6). WT – wild type

xan-b[12]) was significantly lower than that of WT under both salt treatment and the control. This suggested that the plant growth was remarkably limited in the *Xantha* mutants. It was reported that the chlorophyll content in leaves of *xan-b[12]* plants was only 5–50% (depending on the growth temperature) of the WT plants, which reduced the plant biomass (Campoli et al. 2009).

Results of a two-way ANOVA indicated that the g_s was significantly affected by salt treatment, mutation and their interaction (Table 1). Soil salinity reduced the g_s by 48.6, 44.0, 35.3, 37.4 and 24.3% in WT, *xan-s[46]*, *xan-b[12]*, *alb-e[16]* and *alb-f[17]*, respectively (Figure 2). It is interesting that the salt induced reduction in g_s was significantly lower in the *Albina* and *Xantha* mutants than that in WT. This suggested that the sensitivity of stomata in the mutants deficient in magnesium chelatase to soil salinity was lower than WT. Under non-salt conditions, the g_s of the *Albina* and *Xantha* mutants was significantly lower than that of WT, indicating that depressed chloroplast development in *Albina* and *Xantha* mutants negatively affected the stomatal opening, and hence decreased the photosynthetic carbon assimilation and plant growth. It was reported that under high temperature stress, the *Albina* mutant completely lacked and *Xantha* mutant had a significantly decreased photosynthetic activity (Gálová et al. 2000). The chlorophyll *a* fluorescence analysis revealed that heat stress lowered the PS II efficiency of leaves in both WT and mutants (Gálová et al. 2000). In addition, no significant difference was found among *Albina* and *Xantha* mutants both under non-salt and salt conditions.

Soil salinity significantly increased the Na concentration in leaves of WT and mutants (Figure 3).

The concentration of Na was 12.7, 11.5 and 14.0 times higher under salt treatment than that under the control in WT, *xan-s[46]* and *xan-b[12]*, respectively. Whereas, the Na concentration was only 4.6 and 6.8 times higher under salt treatment than the control in *alb-e[16]* and *alb-f[17]* mutants. The lower leaf Na concentration in *Albina* mutants should be related to the lower stomatal conductance in these mutant plants, which resulted in relatively slower xylem transport of ions. However, it should be noted that the leaf K concentration was not affected significantly by salt treatment, but a significant difference was found among WT and mutants. These results suggested that the Na+/K+ homeostasis in barley was changed by not only salt treatment but also the mutations in magnesium chelatase. Numerous studies have documented that a dramatic decline in leaf K concentration was caused by salt stress (Watson et al. 2001, Shabala and Pottosin 2014). However, in this study, the reduction in K concentration caused by soil salinity was not statistically significant in *Albina* and *Xantha* mutants. This may be due to the mutations in magnesium chelatase that weakened the negative effect of soil salinity on K uptake and the consumption of endosperm in barley.
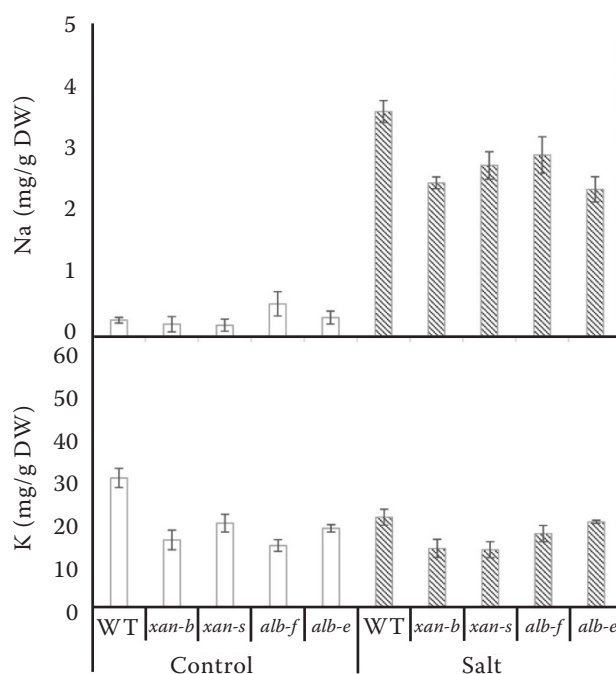

Figure 3. Effects of salt stress on concentrations of Na and K in leaves of barley mutants *Albina* and *Xantha*. Mean values ± standard error are shown (*n* = 6). WT – wild type; DW – dry weight
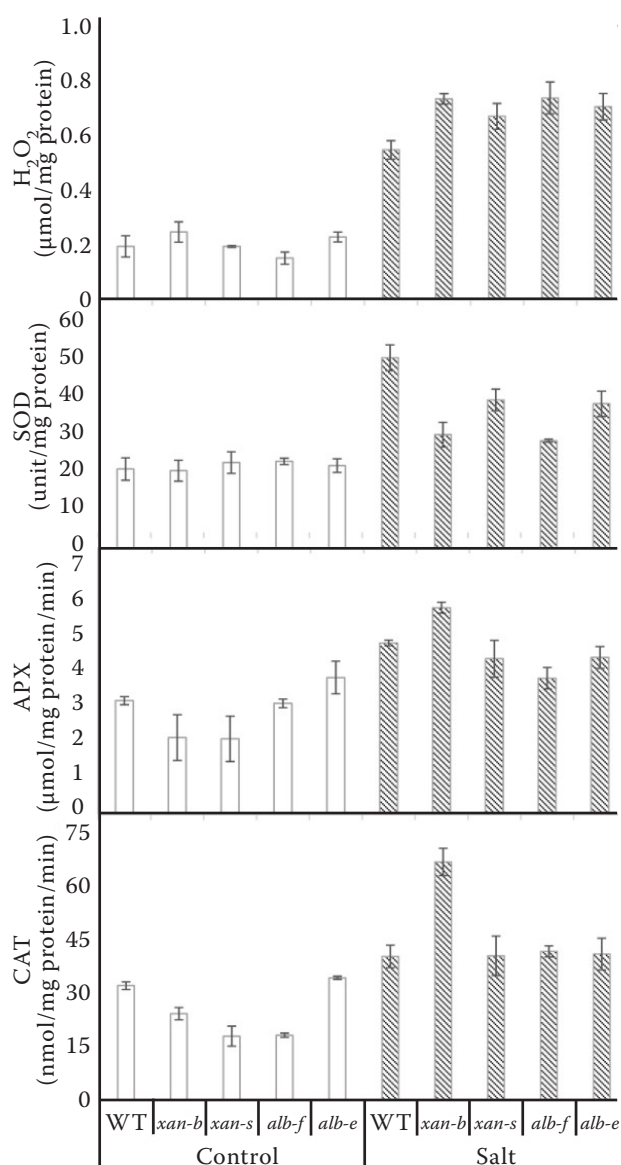
Figure 4. Effects of salt stress on concentrations of H_2O_2 and activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) in leaves of barley mutants *Albina* and *Xantha*. Mean values ± standard error are shown (*n* = 6). WT – wild type

The antioxidant enzyme systems in chloroplasts are important for the oxygen scavenging and ROS balance in plants (Li et al. 2014). In the present study, concentration of H_2O_2 in leaves was significantly increased by soil salinity in both WT and mutants (Figure 4). Under salt treatment, higher H_2O_2 concentration was found in *Albina* and *Xantha* mutants, in relation to WT. This was mainly related to the relatively higher SOD activity in WT compared with the *Albina* and *Xantha*

mutants, exposed to soil salinity. It has been well known that SOD catalyzes the disproportionation of singlet oxygen to produce H_2O_2 in ROS scavenging systems (Keunen et al. 2013). Then CAT and APX decomposes H_2O_2 to H_2O and O_2. Here, exposed to soil salinity, *xan-b[12]* had the highest APX activity in leaf, while no significant difference was found among the WT, *xan-s[46]* and two *Albina* mutants. Also, a similar trend was found in CAT activity, showing the highest value in *xan-b[12]* mutant. The mutations of magnesium chelatase deficiency blocked the chloroplast development, which limited the capacity of subcellular antioxidant systems. However, here, only the activities of APX and CAT in *alb-e[16]* were significantly higher than WT under the control. It was suggested that magnesium chelatase deficiency induced improper chloroplast development did not affect the balance between ROS generation and scavenging in barley under non-stress condition. Under salt
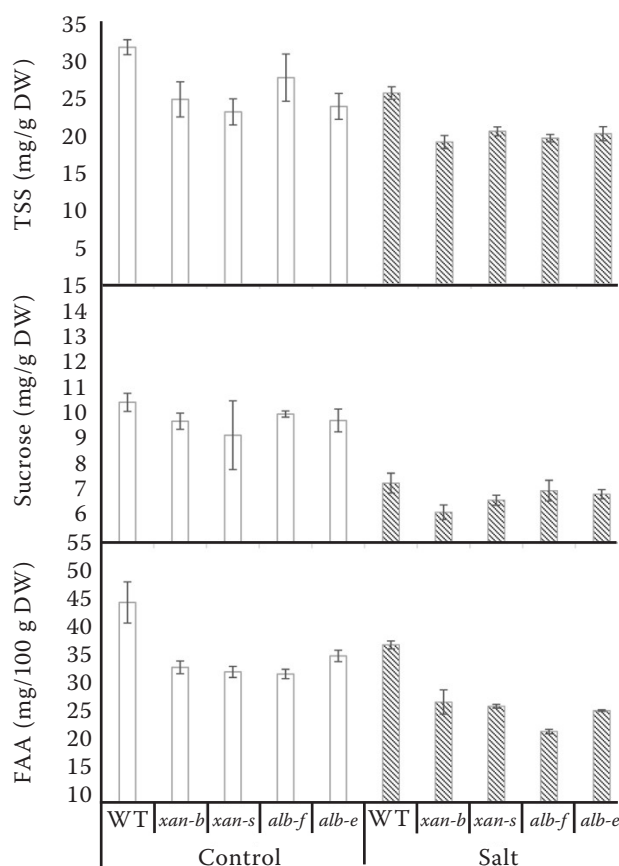

Figure 5. Effects of salt stress on concentrations of total soluble sugar (TSS), sucrose and free amino acids (FAA) in leaves of barley mutants *Albina* and *Xantha*. Mean values ± standard error are shown (*n* = 6). WT – wild type; DW – dry weight

stress, the *Albina* and *Xantha* mutants deficient in magnesium chelatase had lower capacity of ROS scavenging while higher ROS generation rate, compared with WT. It suggested that the sensitivity of barley plants to soil salinity is higher in *Albina* and *Xantha* mutants compared with WT.

The output of the two-way ANOVA indicated that the concentrations of total soluble sugar (TSS) and free amino acid (FAA) were significantly affected by salt treatment, and a significant difference in FAA concentration was found between WT and mutants (Table 1). Soil salinity significantly reduced the concentrations of TSS, sucrose and FAA in both WT and mutants, and the reduction in sucrose was significantly higher in *Albina* and *Xantha* mutants compared with WT (Figure 5). Under salt treatment, lower concentrations of TSS and FAA were observed in *Albina* and *Xantha* mutants compared with WT. It was reported that the photosynthetic carbon assimilation is limited in *Albina* and *Xantha* mutants, which is further depressed by high temperature stress (Hess et al. 1994). In consistency with this, larger reductions in TSS and FAA were found in *Albina* and *Xantha* mutants, compared with WT. This suggested that the soil salinity had a larger effect on the metabolism of carbohydrates in barley *Albina* and *Xantha* mutants deficient in magnesium chelatase, in relation to WT.

In conclusion, soil salinity significantly affected the plant growth in both barley mutants *Albina* and *Xantha* and WT. Larger reduction in g_s and higher Na concentration induced by salt were found in *Albina* and *Xantha* mutants compared with WT, indicating that the sensitivity of barley plants to soil salinity was higher in the mutants deficient in magnesium chelatase. Also, the *Albina* and *Xantha* mutants had lower capacity of ROS scavenging while higher ROS generation rate compared with WT exposed to soil salinity. Therefore, the limitations in chloroplast development affected Na^+/K^+ homeostasis and decreased the oxygen scavenging capacity, hence affecting the salt tolerance in barley.

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